Diseases Caused by Oomycetes

First Report of *Pythium aphanidermatum* Causing Crown and Root Rot on Industrial Hemp in China

Jia Chen,¹ Zhimin Li,¹ Yi Cheng,¹ Chunsheng Gao,¹ Litao Guo,¹ Tuhong Wang,¹ and Jianping Xu^{1,2,†}

¹Institute of Bast Fiber Crops and Center of Southern Economic Crops, Chinese Academy of Agricultural Sciences, Changsha 410205, China

²Department of Biology, McMaster University, Hamilton, L8S 4K1, Canada

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In July 2020, symptoms of crown and root rot were observed on about 10% of 4-month-old plants of industrial hemp (*Cannabis sativa* cultivar Yunma-1) in Weifang City, Shandong Province in eastern China. During this month, the local temperature ranged from 19 to 32°C, and the total precipitation was 148 mm. The disease symptoms included leaf chlorosis, crown and root rot, stunted growth, and wilting. The diseased stem and root tissues were collected and cut into fragments of 0.5 cm each. The fragments were surface sterilized by dipping into 1% NaClO for 1 min, rinsed in sterile water, and plated on potato dextrose agar (PDA) and on oomycete-selective medium PARP (Jeffers and Martin 1986). The plates were incubated at 25°C in the dark for 3 days and 18 total single-hyphal, purified isolates were obtained for further analyses with 8 from PDA and 10 from PARP. The colonies of all 18 isolates were white, had abundant aerial hyphae, and were cottony in appearance, resembling *Pythium* spp. (Watanabe 2002). The grass-leaf method (Van Der Plaats-Niterink 1981) induced their sexual reproduction. The size and shape of hyphae, oogonia, antheridia, and oospores were all consistent with those of *Pythium aphanidermatum*. DNA was extracted from three isolates and their internal transcribed spacer (ITS) regions of ribosomal DNA were amplified and sequenced using the primers ITS1 and ITS4 (White et al. 1990). The ITS sequences of all three isolates were identical to each other (GenBank accession OK091124.1) and showed a 100% query coverage and 99.88% nucleotide sequence identity with that of type strain of *P. aphanidermatum* (GenBank accession AY598622.2). Pathogenicity tests were performed

with three isolates on hemp cultivar B1. Sterile substrates were prepared in 2-liter pots containing peat soil and vermiculite in a 2:1 ratio, with test hemp plants grown from rooted stem cuttings. Plants were kept in a greenhouse at 22 to 27°C under a 16-h photoperiod, watered every 2 days (about 200 ml each time), and supplied commercial nutrient solution once a week. A month after transplanting to pots, a wound 1 mm deep and 10 mm long (made by a sterilized needle) on the surface of the root crown area of the main stem was inoculated with an 8-mm-diameter agar disk of mycelia grown on PDA for 4 days. Six plants were tested for each isolate and three plants were inoculated with sterile agar medium without mycelia as negative controls. The experiment was repeated twice. After 1 month, plants inoculated with *P. aphanidermatum* isolates showed the same disease symptoms as observed on field plants while all negative control plants remained disease free. *P. aphanidermatum* was reisolated from the diseased tissue and confirmed to be identical to those inoculated based on ITS sequencing and colony morphology. To our knowledge, this is the first report of *P. aphanidermatum* causing crown and root rot on hemp in China. With an estimated 66,700 ha of hemp cultivation in China producing over US\$1 billion worth of hemp fiber (McGrath 2020), this pathogen represents a serious threat to the hemp industry. This pathogen has been reported on hemp in the United States and Canada (Beckerman et al. 2017; Punja et al. 2018). The origin of *P. aphanidermatum* on hemp in China and its relationship to species in North America remain to be examined.

References:

- Beckerman, J., et al. 2017. Plant Dis. 101:1038.
- Jeffers, S. N., and Martin, S. B. 1986. Plant Dis. 70:1038.
- McGrath, C. 2020. 2019 Hemp Annual Report. Report no. CH2020-0018. USDA-FAS, Washington, DC.
- Punja, Z. K., et al. 2018. Can. J. Plant Pathol. 40:528.
- Van Der Plaats-Niterink, A. J. 1981. Stud. Mycol. 21:1.
- Watanabe, T. 2002. Pictorial Atlas of Soil and Seed Fungi. CRC Press, Boca Raton, FL.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

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[†]Indicates the corresponding author.
J. P. Xu; jpxu@mcmaster.ca